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Microarray Method for the Rapid Detection of Glycosaminoglycan–Protein Interactions

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Abstract

Glycosaminoglycans (GAGs) perform numerous vital functions within the body. As major components of the extracellular matrix, these polysaccharides participate in a diverse array of cell-signaling events. We have developed a simple microarray assay for the evaluation of protein binding to various GAG subclasses. In a single experiment, the binding to all members of the GAG family can be rapidly determined, giving insight into the relative specificity of the interactions and the importance of specific sulfation motifs. The arrays are facile to prepare from commercially available materials.

Keywords

Glycosaminoglycan; Chondroitin sulfate; Dermatan sulfate; Heparan sulfate; Heparin; Hyaluronic acid; Microarray; Growth factor; Glycosaminoglycan-binding protein

1. Introduction

Glycosaminoglycans (GAGs) are a large family of linear polysaccharides that fulfill diverse functions in vivo, such as joint lubrication and movement (1), cell signaling and development, angiogenesis (2), axonal growth (3), viral invasion (4), spinal cord injury (5, 6), tumor progression (7, 8), metastasis (7, 9), and anti-coagulation (10, 11). GAGs are large (typically 10–100 kDa), highly charged, and heterogeneously sulfated molecules composed of repeating disaccharide units. Members of the GAG family vary subtly in stereochemistry, length, and sulfation pattern (Fig. 1a). For instance, chondroitin sulfate (CS), the most abundant GAG in the body is composed of the repeating disaccharide D-glucuronic acid (GlcA) and *N*-acetyl-D-galactosamine (GalNAc). CS is further classified by the sulfation pattern of its disaccharides, the most common of which are termed CS-A, -C, -D, and -E (Fig. 1b). Dermatan sulfate (DS), also known as CS-B, differs from CS in the stereochemistry of the *C*-5 position of the uronic acid. Heparin and heparin sulfate (HS) are composed of D-glucosamine (GlcN) and either GlcA or its *C*-5 epimer L-iduronic acid (IdoA). The GlcN can either be *N*-sulfated, protonated, or acetylated. In general, HS has more GlcA and *N*-acetylated GlcN then heparin, and heparin has a much higher charge density and more *N*-sulfated GlcN than HS. Keratan sulfate (KS) is composed of D-galactose and *N*-acetyl-D-glucosamine (GlcNAc) and is the only GAG that does not contain uronic acid. Hyaluronic acid (HA), the only unsulfated GAG, is composed of GlcA and GlcNAc.

The chemical diversity of GAGs is believed to have important functional consequences, enabling a large number of protein-binding motifs to be generated from a relatively simple scaffold (12, 13). For instance, HS is important for growth factor signaling, inflammation, and blood coagulation (10, 11, 14, 15), while CS has been shown to interact with various growth factors involved in stem cell proliferation, neurogenesis and gliogenesis, and is a major component of the glial scar, an inhibitory barrier that forms after spinal cord injury (6, 16).

A major challenge in understanding GAG function has been the lack of high-throughput methods to identify protein–GAG interactions. While effective, methods such as affinity chromatography, electrophoretic mobility shift assays, competition experiments, mass spectrometry-based approaches, isothermal titration calorimetry, and surface plasmon resonance are frequently labor intensive and require significant quantities of carbohydrate and/or protein. Given the diverse structure of GAGs and the large number of potential protein-binding motifs, a high-throughput approach for the discovery and study of protein–GAG interactions is needed. Moreover, the highly anionic character and other structural similarities among GAGs necessitate a method to compare the relative affinities of proteins for different GAG family members and for different sulfation patterns within a GAG class.

The recent development of GAG microarrays (17–21) has enabled many of these challenges to be addressed. Microarrays allow for the rapid, simultaneous detection of multiple protein–GAG binding events and require minimal amounts of carbohydrate and protein. Methodologies have been developed for studying the binding of growth factors, cell-surface receptors, and chemokines to sulfated variants of CS and HS (17, 19, 21) and for comparing the binding specificities of proteins across various GAG classes (18, 20). Microarrays have been constructed using chemically synthesized CS and HS oligosaccharides, which have the advantage of defined sulfation patterns (17, 19, 21), or from naturally occurring polysaccharides (18, 20). Here, we describe a microarray-based approach for the study of protein–GAG interactions that employs commercially available sugars and simple adsorption to affix the sugars to the array surface. The microarrays are relatively inexpensive, easy to prepare, and enable the rapid evaluation of protein-binding specificities across the entire GAG family in a single assay.

2. Materials

2.1. Slide Preparation

1. Microslides (25 × 75 × 1.0 mm, VWR; West Chester, PA, see Note 1).
2. Phosphate-buffered saline (PBS): Prepare 10× stock with 1.37 M NaCl, 27 mM KCl, 54 mM Na_2HPO_4, 18 mM KH_2PO_4, pH 7.4. Dilute 100 mL of 10x stock with 900 mL of water for use (see Note 2).

[1]Alternatively, we recommend the poly-L-lysine-coated slides from Erie Scientific, Portsmouth, NH. If pre-coated slides are used, ignore Subheading 3.1 and start the procedure at Subheading 3.2.

[2]Throughout the text, “water” refers to water that has a resistivity of 18.2 MΩ cm and total organic content of less than five parts per billion.

3. Glass staining dishes with removable racks (105 × 70 × 85 mm, Wheaton Science Products, Millville, NJ).
4. Poly-L-lysine solution: combine 80 mL of 0.1% (w/v) poly-L-lysine solution in H_2O with 80 mL of PBS and 640 mL of water.
5. Etch solution: dissolve 150 g solid NaOH in 600 mL of water. Mix in 900 mL of 95% ethanol (see Note 3).
6. Slide box. Prior to use, blow compressed air into the box to remove any dust particles.
7. Chondroitin sulfate A, C, D, E (Seikagaku America; Fallmouth, MA) are dissolved at 500 μM in water and stored at 4°C (see Note 4).
8. Chondroitin sulfate B (known also as dermatan sulfate, Sigma-Aldrich; St. Louis, MO) is dissolved at 500 μM in water and stored at 4°C.
9. Hyaluronic acid (Sigma-Aldrich; St. Louis, MO) is dissolved at 500 μM in water and stored at 4°C.
10. Heparin polysaccharide VI (Neoparin; Alameda, CA) are dissolved at 500 μM in water and stored at 4°C.
11. Keratan sulfate (Seikagaku America; Fallmouth, MA) is dissolved at 500 μM in water and stored at 4°C.
12. High sample recovery 384-well plate and covers (Genetix; Boston, MA).
13. Microplate sealing film (VWR; West Chester, PA).
14. Lint-free paper: Bluesorb 750, 4 × 4, non-woven polyester/cellulose (Berkshire; Surrey, UK).
15. 70% Ethanol solution: Dilute 37 mL of 95% ethanol with 13 mL of water.
16. Microgrid II (Biorobotics; Cambridge, UK) or other suitable arrayer.

2.2. Carbazole Assay

1. Acid borate reagent: A solution of 0.80 g sodium tetraborate dissolved in 16.6 mL of water and 83.3 mL of sulfuric acid is stored at room temperature.
2. Carbazole reagent: 0.1% (w/v) carbazole is dissolved in 100% ethanol, protected from light and stored at 4°C.
3. Glucuronolactone standard: D-Glucuronic acid lactone (Sigma-Aldrich; St. Louis, MO) at 1 mg/mL in water, stored at 4°C.

[3] *Caution*: Wear lab coat and safety glasses when preparing this solution. This solution becomes hot when the reagents are mixed. Ensure the solution is carefully vented if mixing in a sealed container.

[4] Due to the heterogeneity of GAG samples in terms of chain length, degree of sulfation, and number and type of counter ions (both within a sample and between different GAGs), the molecular weights for each sample are only approximate. Prepare 500 μM samples based on the average molecular weight for each sample. To compare different GAGs to one another, we measure the average uronic acid concentration for each sample using the carbazole assay described in Subheading 3.

2.3. Protein Binding Assay

1. Super Pap Pen (Research Products International Corp.; Mount Prospect, IL).
2. Blocking buffer: 3% (w/v) bovine serum albumin (BSA) in PBS. Alternatively, 10% (w/v) fetal bovine serum (FBS) in PBS can be used. In either case, filter through a 0.20 μm cellulose nitrate membrane and store at 4°C.
3. Protein dilution buffer: PBS supplemented with 1% (w/v) BSA, filtered through a 0.20 μm cellulose nitrate membrane and stored at 4°C.
4. Protein(s) of interest. For example: Recombinant human beta-NGF (Peprotech; Rocky Hill, NJ).
5. Incubation box: DVA211 6-compartment plastic box (7 × 3.75 × 1.25 in., Durphy Packaging Co.; Ivyland, PA).
6. Primary antibody: Primary antibody/antibodies against protein of interest at 1 mg/mL. For example: Rabbit anti-human β-NGF (Peprotech; Rocky Hill, NJ).
7. Secondary antibody: Cy3- or Cy5-conjugated secondary antibody at 1 mg/mL (see Note 5). For example: Cy3 goat anti-rabbit IgG (Invitrogen; Carlsbad, CA).
8. GenePix 5000a scanner with GenePix 6.1 software (Affymetrix; Fremont, CA).

3. Methods

The carbohydrate microarray methodology described herein exploits the high charge density of GAGs to affix the sugars to the array surface. Adsorption is simple and effective and allows GAGs to be used directly, without additional modification. As members of the GAG family can vary considerably in length, GAG concentration must be determined in terms of uronic acid concentration. It is necessary to normalize binding data with respect to the uronic acid concentration because longer GAG molecules may have more binding sites per mole.

The carbohydrate microarray methodology is robust and provides reproducible and consistent results. The microarrays are very sensitive and even weak protein–GAG interactions (e.g. K_D > 10 μM) can be detected. Therefore, it is important to interpret results with caution and use independent methods to confirm that the observed binding is strong and physiologically relevant. Carbohydrate microarrays provide a powerful, rapid method to screen for novel protein–GAG interactions, but as with any method, they must be used in combination with other techniques. Furthermore, care should be taken when comparing the relative affinity of a given protein–GAG interaction to another based on the difference in fluorescence intensity between two microarrays. The difference could be due to number of factors and does not necessarily reflect a difference in affinity. A quantitative assay should be used to compare differences in affinity from protein to protein.

[5]The choice of Cy3 and Cy5 dyes was based on the scanner wavelengths of the GenePix 5000a scanner. If using a different scanner, check the manufacturer's specifications and use dyes compatible with the instrument's filters.

3.1. Preparation of Poly-L-Lysine-Coated Slides (See Note 1)

1. Place the microslides into the removable racks of the staining dishes. Examine each slide, checking for markings that cannot be removed by Kim-Wipes. Place 19 slides in each rack and place the rack into the empty dish (see Note 6).
2. Carefully pour approximately 200 mL of the etch solution into the dishes (see Note 7). Make sure that the slides are completely covered. Cover the dish with the lid and incubate the slides in the etch solution for 1 h.
3. Remove the etch solution (see Note 8), and rinse the slides in the dishes five or more times in approximately 200 mL of water for approximately 10 s, moving the rack in an up-and-down motion at a constant and consistent speed. It is critical that all of the etch solution is removed before continuing to the next step.
4. Pour approximately 200 mL of the poly-L-lysine solution into each dish, making sure the slides are covered. Place the dishes on an orbital shaker at a speed low enough that none of the poly-L-lysine solution will splash out. Incubate with shaking for 1 h.
5. Remove the poly-L-lysine solution and rinse the slides with water as described in Subheading 3.1, step 3 above. After the final rinse, leave the slides in water for the next step.
6. One dish at a time, remove the rack from the water and dry the rack and the slides under a stream of compressed air to remove most of the water. Then, without touching the surface, dry the slides individually under a stream of compressed air, making sure the slides are completely dry. Place the slides into the slide box (see Note 6). Once all the slides have been dried and transferred to the slide box, label and date the box, and place the box in a desiccator. Allow at least 2 weeks before printing the slides to ensure complete dryness.

3.2. Preparation of Sugar Samples

1. Make a series of samples containing 0, 1, 3, 5, 7.5, and 10 μL of the glucuronolactone standard. Adjust the final volume to 50 μL with water.
2. For each GAG, prepare 1, 3, and 5 μL samples from the 500 μM stocks. Adjust the final volume to 50 μL with water.
3. For each sample (both the glucuronolactone and GAG dilutions, 33 samples in total), add 1 mL of acid borate reagent to a test tube, followed by the 50 μL samples prepared in Subheading 3.2, steps 1 and 2. Mix by vortexing, cover each tube with foil or parafilm, and place the samples in a boiling water bath for 10 min.

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[6] Wear gloves whenever handling the slides. Make sure the slides are arranged in the rack such that both sides of the slide are exposed to solution.

[7] *Caution*: Wear lab coat and safety glasses when handling the etch solution.

[8] It is possible to reuse the etch solution. The solution is good for up to 1 month, although if discoloration is observed, the solution should be remade.

4. After cooling the samples to room temperature, add 50 μL of carbazole reagent, mix by vortexing, cover, and return the mixtures to the boiling water bath for an additional 15 min.

5. After cooling the samples, measure the absorbance at 530 nm. For the D-glucuronolactone standards and the GAG samples, plot volume of stock used versus absorbance, and determine the slope of the resulting curve using linear regression analysis. Determine the molarity of each sample by dividing the slope of the GAG dilution series by that of the D-glucuronolactone standard, then divide the quotient by the average molecular weight of the GAG.

3.3. Printing Slides

1. These instructions assume the use of a Microgrid II arrayer. If using another instrument to print arrays, follow the manufacturer's instructions. It is critical that the arrays have multiple replicates of each concentration of GAG and that the spot morphology is consistent. Maintaining the dimensions of the array and the location of the GAGs within the array is less important.

2. Using the concentration of the GAG samples determined in Subheading 3.2, step 5, prepare 15 μL of 0.5, 1, 2, 5, 10, 15, 20 μM samples of each GAG in water from the standardized stocks.

3. Place the samples into the high sample recovery 384-well plate. Start filling the plate at well A1, and fill the remaining wells such that a minimal number of 4 × 4 grids are filled. For example, if 16 samples are used, fill the wells A1-D4, inclusive. For these arrays, with seven concentrations of nine GAGs, use the wells between A1 and P4, with one well empty as a blank. Cover the plate with the microplate sealing film and lid and store at 4°C until use. Record the location of each sample, including the blank, in an Excel spreadsheet. Export the file as a tab delimited text file. If using an operating system that uses end-of-line characters (EOLs) different from the Windows operating system (such as Unix-based systems, including Mac OS X), change the EOLs to be Windows compatible (see Note 9).

4. Transfer the tab separated text file with Windows compatible EOLs to the computer that operates the Microgrid II Arrayer. Open the TAS application suite program on this computer to set up the print run.

5. From the file menu, select “New Microarray.” In the new window that appears, under the “Options” tab, under “Group:” select “2. MicroSpot (384 well)” and under “Tool:” select “4 × 4 configuration.”

6. Click the “Source” tab. Under “Microplate Group”: select “Generic,” under “Microplate Type”: select “384 well (low profile),” under “Number of Plates”: type 1. Confirm the “Number of Samples” matches the number of samples in the 384-well plate in Subheading 3.3, step 2. Under “Lid Removal” check “Replace lid

[9]If using Mac OSX, it is possible to convert the tab separated file to be windows compatible by using the following command in Terminal:

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tr '\r' '\n' < inputfile > outputfile.
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immediately." Select "Remove one lid at a time." Under "Source action" select "dwell."

7. Under the "Target" tab, under "Tool array definition" change the "size" to be 6 × 5, and the "pitch" to be 0.500 mm.

8. Under the "Format" section, select the "n" radio button and enter "10" for the number of replicates to print and edit the location of each replicate within the print block in the "Edit" window. After the layout is saved, the selected radio button will become "Custom." Under "Adapter Plate and Slide Layout," enter the number of slides to print in the "targets" field.

9. Enter the dimensions of the array. Press the "Slide layout" button. Make sure the option "Mirror vertical margins" option is unchecked. Enter 18.15 mm for the top margin, and 12.90 mm for the bottom margin (see Note 10). Check the "Mirror horizontal margins" options and type 3.40 mm for Left margin, 0.00 mm for *x* spacing, and 11.00 mm for *y* spacing. The resulting array will have two identical array regions per slide with dimensions as shown in Fig. 2. A representative array with sample GAG concentrations is depicted. The concentrations, GAGs and layout of the array can be tailored to the protein of interest.

10. Under the "Target action" tab, type 0s under "Delay before spotting," 0.6 mm under "Target Height," 0s under "Dwell time," 1 under "Multiple strikes." Make sure the "soft touch" option is checked and that the "Pre-spotting" option remains unchecked.

11. Close this window, when prompted save the method.

12. From the file menu of the TAS application suite program, select "Clone tracking wizard…" Click "Next" twice, then select "No, plates do not have barcodes," and then click "Next" again. In the type of output file dialog window, select "I already know what file type I need" and choose "Axon GAL" from the pull-down menu. Select the "Import name and ID" option and type 80 μm under the field labeled "Typically the spots I am printing are." Click "Next," and then select the tab delimited text file with Windows compatible EOLs that was saved on the computer in Subheading 3.3, step 3. Check the "Tab" option below.

13. After clicking "Next," the wizard will display the contents of the imported text file. Confirm that the imported file is correct and that there are no errors. Click "Next." Check again that the file is correct, if so, press "Output file." Give the file a name and select a location to save it. Click "Save," then "Next." Transfer this file to the computer that runs the GenePix Sanner.

14. Select 16 pins from the Microgrid II arrayer accessories. Make sure that the pins are not bent or damaged in any way. Submerge the tips of the pins in 15 mM KOH in water. After 5 min, remove the pins from this solution and sonicate the tips of the pins for 5 min, while submerged in 0.01% Tween-20 in water. Rinse the pins by

[10]The asymmetric margins will help determine the proper orientation of the slide if necessary.

submerging the tips in water and sonicating for 5 min. Replace the water and repeat two more times. Rinse the pins by dipping them in 95% ethanol in water and place them on the lint-free paper to dry (see Note 11).

15. Fill the large bottle supplied with the arrayer with water. Click the “Fill 6-litre reservoir” icon on the TAS application suite program. The progress can be monitored with the icon to the left of this button. Turn on the recycling water bath pump and wait for the coolant temperature to drop to 8°C (approximately 30 min).

16. Under the “Housekeeping” menu in the TAS application suite program, click “load/unload tray 1.” Clean tray with compressed air to make sure that it is dust free. Carefully place the poly-L-lysine-coated slides onto the tray after checking that they are dust free. Remove any dust with a stream of compressed air, if needed. Continue loading slides into the remaining trays if necessary. Each tray must contain exactly 30 slides. If printing a number of slides that is not divisible by 30, use dust-free plain glass slides to fill the remaining slots in the tray. Nothing will be printed on these slides, but they are necessary to maintain the vacuum applied to the tray to keep the slides in place during printing.

17. Select the “Load tool” option under the “Housekeeping” menu. Load the clean and dry pins in the orientation shown by the wizard.

18. Next, select “Load biobank” from the “Housekeeping” menu. After removing the film and placing the cover on the 384-well sample plate, place into machine.

19. On the bottom panel of the chamber in the robot, there should be three reservoirs. Fill the left-hand reservoir with water and the middle with 70% ethanol in water. Lastly, to maintain humidity in the chamber, take three 384-well plate lids, and place a few paper towels into each lid, cutting them to fit as necessary. Fill the lids with water, making sure the paper towels are saturated. Place the lids on the bottom of the chamber. Close the chamber lid, and press the “GO” icon in the TAS application suite program. This will initiate printing.

20. When the printing is finished, unload the slides via the wizard in the “Housekeeping” menu. Transfer slides into a dust-free slide box. Label the top-right corner of the slide using a diamond-tipped pen (see Note 6). Store arrays in a low-humidity, dust-free desiccator.

21. Unload pins via the wizard in the “Housekeeping” menu and repeat the cleaning procedure detailed in Subheading 3.3, step 13.

22. Remove the 384-well sample plate via the wizard in the “Housekeeping” menu. If sufficient volume remains, the plate can be resealed with film, covered and stored at −20°C for an additional print run.

23. Drain the reservoir and shut down the robot.

[11]Handle the pins very carefully and only with tweezers.

3.4. Protein Binding Assay

1. Using a hydrophobic marker, such as a PapPen, draw a perimeter around the printed region of the slide according to the dimensions for the array region given in Subheading 3.3, step 8. This perimeter reduces the amount of protein required to fully cover the array region. However, take care not to mark the slide too close to the printed region, leaving up to 0.5 cm of space when possible. This is important because the hydro-phobic marker can prevent the protein from interacting with the carbohydrate spots near the edge of the array.
2. Place the slide in the incubation box and cover the slide with 2.5 mL of blocking buffer at 37°C for 1 h with gentle rocking. This step is necessary to prevent nonspecific interactions between the proteins and the surface of the array.
3. Remove the blocking buffer and add the protein sample (0.5-2 μM in protein dilution buffer, see Note 12) to the printed region of the slide. Make sure that the slide does not dry out before adding the protein. Also, make sure that there are no water “bridges” over the hydrophobic pen markings. If so, carefully blot dry with a Kim-Wipe. Be sure to add sufficient volume to fully cover the region (100–200 μL). Incubate at room temperature for 1–3 h.
4. Wash the slide five times for 30 s each with 2.5 mL PBS with gentle rocking.
5. Incubate the slide in 2.5 mL of a 1:1,000 or appropriate dilution (see Note 13) of primary antibody in protein dilution buffer for 1 h at room temperature with gentle rocking. Alternatively, 100–200 μL of the antibody solution can be added to the array region as described in Subheading 3.4, step 3.
6. Remove the antibody solution and wash the slide five times for 30 s each with 2.5 mL PBS with gentle rocking.
7. Incubate the slide in 2.5 mL of a 1:5,000 or appropriate dilution (see Note 13) of secondary antibody in protein dilution buffer for 1 h at room temperature with gentle rocking.
8. Remove the antibody solution and wash the slide three times for 30 s each with 2.5 mL PBS and two times for 30 s each with 2.5 mL water with gentle rocking.
9. Immediately after the final wash step, dry the slide(s) under a gentle stream of air or nitrogen. This prevents water droplets from evaporating on the array, which could potentially obscure the signal.
10. (Optional) Add a droplet (~5 μL) of a fluorescence-specific mounting medium, such as VectaShield, to the printed area of the array. Carefully place a coverslip

[12]When testing a protein with unknown affinity to GAGs, a good starting concentration is 2 μM, although less protein can be used if the sample is precious. Some proteins have very high affinity to GAGs and will saturate the signal when incubated at 2 μM, even when scanned at extremely low laser power. If this is the case, it is necessary to reduce the concentration of protein to obtain useful data.

[13]When using unknown antibodies, a good starting dilution is 1:1,000 for the primary antibody and 1:5,000 for the secondary antibody. However, particularly strong antibodies may require a higher dilution, and weak antibodies may require a lower dilution.

over the drop, taking care to avoid forming any bubbles, and seal the coverslip with nail polish.

3.5. Recording Data

1. These instructions are specific for the GenePix 5000a scanner using GenePix 6.1 software. They are easily adaptable to other microarray scanners. Follow the manufacturer's instructions. It is critical that the dye on the secondary antibody is compatible with the filters on the scanner (see Note 5) and that the array is scanned using appropriate laser power and gain.
2. On the computer controlling the GenePix 5000a scanner, open the GenePix 6.1 software and wait for the scanner to initialize.
3. Place the slide into the GenePix 5000a scanner. Orient the slide such that the printed region of the slide is facing down and the top of the array is pointed into the scanner. If the slides were labeled according to Subheading 3.3, step 19, the label will be in the back left of the scanner.
4. In the GenePix 6.1 program, click the “Hardware Settings” icon. In the menu that appears, there will be two fields labeled “Select Wavelength.” Select one by clicking the checkbox on the left, and make sure the other is not checked. Under the pull-down menu to the right of the “Select Wavelength” field, select the wavelength used for the experiment (532 nm for Cy3 or 635 nm for Cy5). Under “PMT Gain”: enter the desired value for the gain. A reasonable place to start is 400. Under “Power (%)”: begin at a low percent power, such as 5–10%. Under “Filter”: select the corresponding filter for the wavelength (“Standard Green” for 532 nm/Cy3 or “Standard Red” for 635 nm/Cy5). Lastly, change “Pixel Size (μm)” to 5, “Lines to Average” to 1, and “Focus Position” to 0, if necessary.
5. Click the “Preview Scan” icon. This will take a quick scan of the array. Adjust the brightness and contrast in the Tools section if necessary. While it may not be possible to distinguish the signal from the background at this resolution, the preview scan is helpful for determining whether the PMT Gain or Power needs to be increased or decreased. If so, repeat the preview scan.
6. If the preview scan is acceptable, click the “View Scan Area” icon under the Tools panel. Using the mouse, highlight the array region of the slide. A white rectangle will appear. Resize or move the rectangle with the mouse if necessary.
7. When the array region of the slide is within the area delimited by the rectangle, click the “Data Scan” icon. This takes a high-resolution image of the array region of the slide. After the scan has finished, click the “File…” icon and select “Save Images.” After saving, zoom into the array region to see the signal, which should look like small, ordered spots. Adjust the brightness and contrast as needed, or rescan the image after adjusting the PMT Gain and Power if necessary. The image should look similar to Fig. 3a, b.
8. If the data scan is acceptable, click the “View Blocks” icon. Then, under the “File…” menu, click “Load Array List…” Find the .gal file that was created in

Subheading 3.3, steps 11 and 12 and click “Open.” A series of boxes with small circles inside will appear. Using the mouse, select all of the boxes and move them roughly into position (i.e., over the spots corresponding to protein bound to GAG). To more precisely position the blocks, select one block at a time, zoom into the region, and move the block such that the spots (i.e., the signal) are centered in the circles. When the ideal adjustment has been achieved, press F5. Repeat for the remaining blocks (see Note 14).

9. Click the “Analyze” icon. Under the “File…” menu, click “Save Results As…” Name the file and click “Save.” This .gpr file can be opened in Excel and analyzed, as in Fig. 3c.

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[14]If the program has difficulty adjusting the grid to the signal, right click on a box and select “Block Properties,” and adjust the diameter of the circles accordingly.

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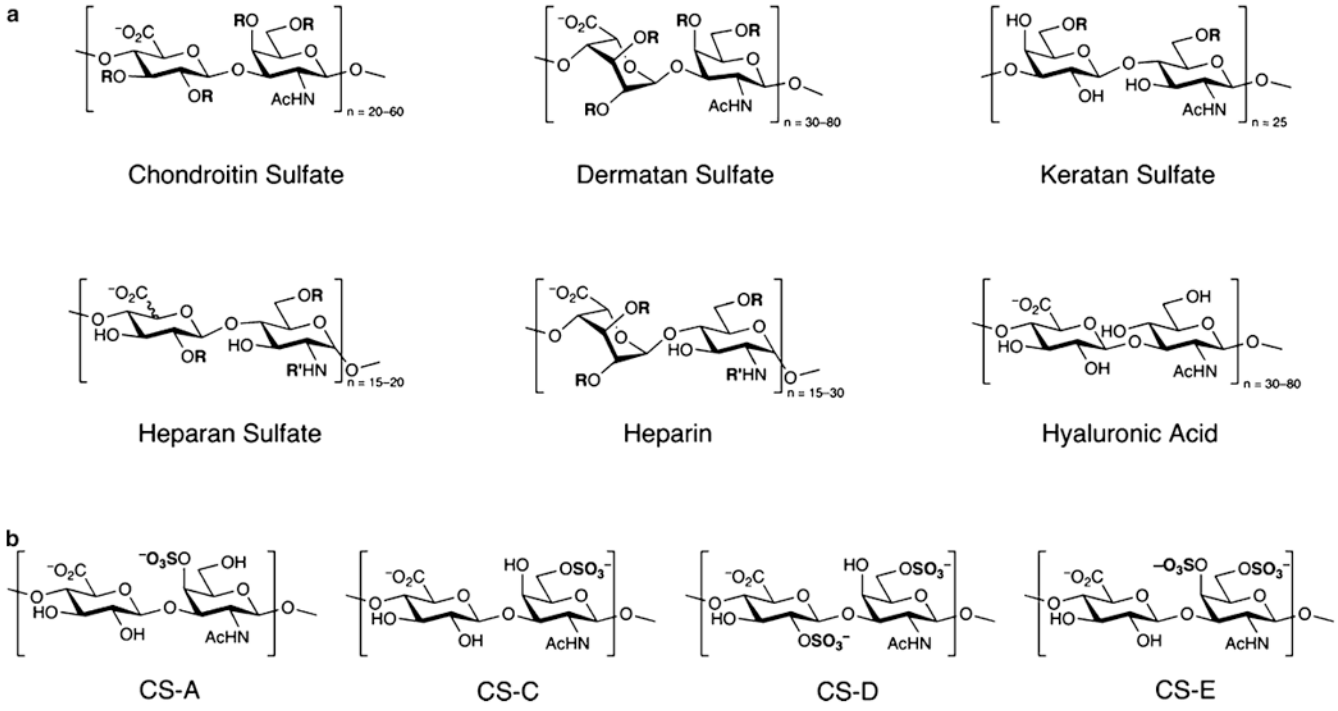

Fig. 1.

(**a**) Structures of glycosaminoglycan family members. Indicated hydroxyl groups can either be protonated or sulfated (R = H or SO_3^-). Indicated amino groups can be either protonated, acetylated, or sulfated (R′ = H, Ac, or SO_3^-). (**b**) The common CS sulfation patterns.

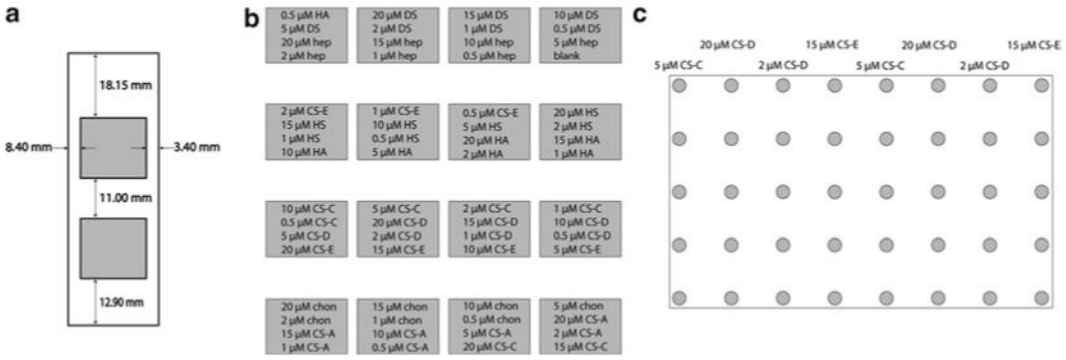

Fig. 2.

(**a**) The dimensions of the array on the 25 × 75 mm slide. The *gray boxes* represent the array regions. (**b**) Detail of the array regions from (**a**). The array features 16 blocks with ten replicates of four concentrations of GAGs. The concentrations and GAGs are labeled within each block. (**c**) A detail of the layout of a block. This is the block in the *third row* from the *top*, *second column* from the *left*.

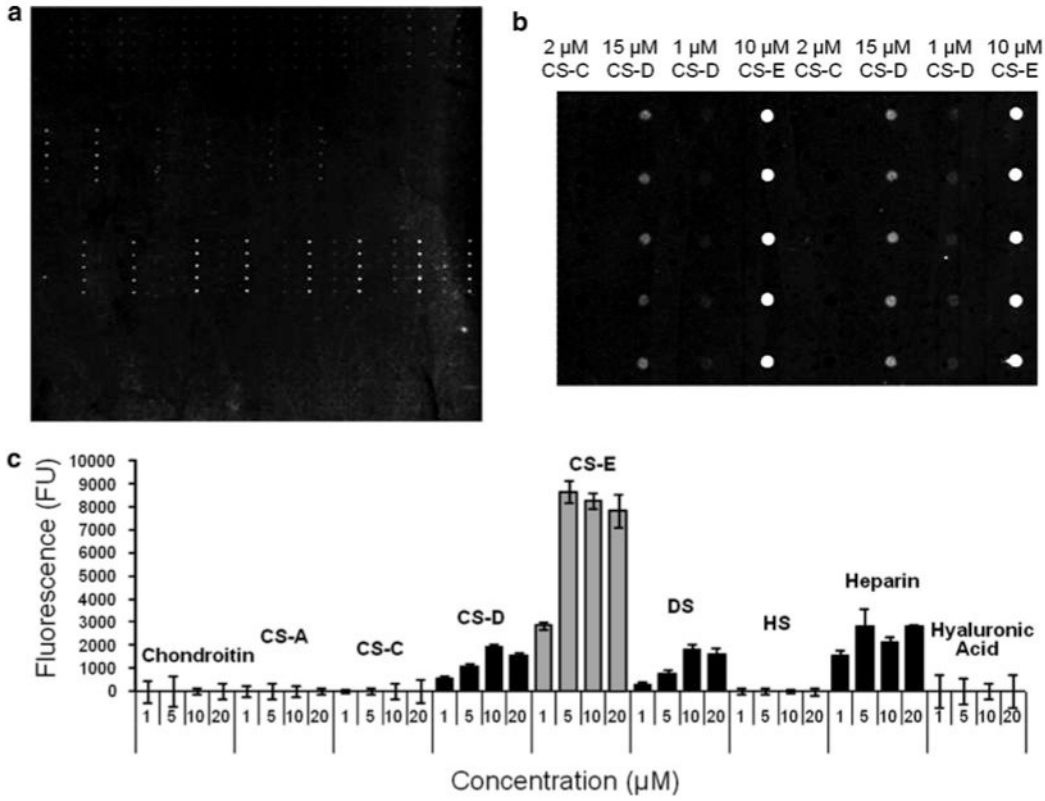

Fig. 3.

(**a**) A representative image of nerve growth factor (NGF) binding to a GAG microarray as visualized using a Cy3-conjugated secondary antibody against anti-NGF. (**b**) An expansion of a region of the microarray from (**a**). The columns, from *left* to *right*, in (**b**) are 2 μM CS-C; 15 μM CS-D; 1 μM CS-D; 10 μM CS-E; 2 μM CS-C; 15 μM CS-D; 1 μM CS-D; 10 μM CS-E. Each concentration is repeated five times down the column. (**c**) Quantification of the data in (**a**) for binding of NGF to various GAG subclasses.